

UCSF

UC San Francisco Previously Published Works

Title

Genomic profiling of malignant peritoneal mesothelioma reveals recurrent alterations in epigenetic regulatory genes BAP1, SETD2, and DDX3X.

Permalink

<https://escholarship.org/uc/item/3qb2r7q5>

Journal

Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc, 30(2)

ISSN

0893-3952

Authors

Joseph, Nancy M
Chen, Yunn-Yi
Nasr, Anthony
et al.

Publication Date

2017-02-01

DOI

10.1038/modpathol.2016.188

Peer reviewed



Published in final edited form as:

Mod Pathol. 2017 February ; 30(2): 246–254. doi:10.1038/modpathol.2016.188.

Genomic Profiling of Malignant Peritoneal Mesothelioma Reveals Recurrent Alterations in Epigenetic Regulatory Genes *BAP1*, *SETD2*, and *DDX3X*

Nancy M. Joseph^{1,2}, Yunn-Yi Chen¹, Anthony Nasr³, Iwei Yeh^{1,2}, Eric Talevich², Courtney Onodera², Boris C. Bastian^{1,2}, Joseph T. Rabban¹, Karuna Garg¹, Charles Zaloudek¹, and David A. Solomon^{1,2}

¹ Department of Pathology, University of California, San Francisco, CA

² Clinical Cancer Genomics Laboratory, University of California, San Francisco, CA

³ Pathology Sciences Medical Group, Chico, CA

Abstract

Malignant mesothelioma is a rare cancer that arises from the mesothelial cells that line the pleural cavity and less commonly from the peritoneal lining of the abdomen and pelvis. Most pleural mesotheliomas arise in patients with a history of asbestos exposure, whereas the association of peritoneal mesotheliomas with exposure to asbestos and other potential carcinogens is less clear, suggesting that the genetic alterations which drive malignant peritoneal mesothelioma may be unique from those in pleural mesothelioma. Treatment options for all malignant mesotheliomas are currently limited, with no known targeted therapies available. To better understand the molecular pathogenesis of malignant peritoneal mesothelioma, we sequenced 510 cancer-related genes in 13 patients with malignant mesothelioma arising in the peritoneal cavity. The most frequent genetic alteration was biallelic inactivation of the *BAP1* gene, which occurred in 9/13 cases, with an additional 2 cases demonstrating monoallelic loss of *BAP1*. All 11 of these cases demonstrated loss of BAP1 nuclear staining by immunohistochemistry, whereas the 2 tumors without *BAP1* alteration and all 42 cases of histologic mimics in peritoneum (8 multilocular peritoneal inclusion cyst, 6 well-differentiated papillary mesothelioma of the peritoneum, 16 adenomatoid tumor, and 12 low-grade serous carcinoma of the ovary) demonstrated intact BAP1 nuclear staining. Additional recurrently mutated genes in this cohort of malignant peritoneal mesotheliomas included *NF2* (3/13), *SETD2* (2/13), and *DDX3X* (2/13). While these genes are known to be recurrently mutated in pleural mesotheliomas, the frequencies are distinct in peritoneal mesotheliomas, with nearly 85% of peritoneal tumors harboring *BAP1* alterations versus only 20-30% of pleural tumors. Together, these findings demonstrate the importance of epigenetic

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: http://www.nature.com/authors/editorial_policies/license.html#terms

To whom correspondence should be addressed: Nancy M. Joseph, MD, PhD, Department of Pathology, University of California, San Francisco, 1600 Divisadero Street, Room B-620, Box 0785, San Francisco, CA 94143, Ph: 415-502-3119, nancy.joseph@ucsf.edu, - or-, David A. Solomon, MD, PhD, Department of Pathology, University of California, San Francisco, 513 Parnassus Ave, Box 0102, Health Sciences West 451, San Francisco, CA 94143, Ph: 415-514-9761, david.solomon@ucsf.edu.

Conflicts of interest: Nothing to disclose.

modifiers including *BAP1*, *SETD2*, and *DDX3X* in mesothelial tumorigenesis and suggest opportunities for targeted therapies.

Keywords

Malignant peritoneal mesothelioma; well-differentiated papillary mesothelioma; benign multicystic mesothelioma; adenomatoid tumor; low-grade serous carcinoma; *BAP1*; *NF2*; *SETD2*; *DDX3X*

INTRODUCTION

Malignant mesothelioma is a rare cancer that arises from the mesothelial cells that line the pleural cavity (>80% of cases) and less commonly from the peritoneal lining of the abdominal and pelvic cavities (10-15%), the pericardial lining, and the tunica vaginalis. Approximately 2,500 malignant mesotheliomas are newly diagnosed in the United States each year, which have a uniformly poor prognosis and median survival time of less than two years. Greater than 80% of malignant pleural mesotheliomas arise in patients with a history of asbestos exposure, whereas malignant peritoneal mesotheliomas have less clear risk factors that include chronic peritonitis.¹ Patients with malignant peritoneal mesothelioma are more commonly female, of younger age, and have better prognosis than patients with pleural mesothelioma, though most patients will eventually die of disease.²

Genomic analysis has defined the spectrum of molecular alterations that drive pleural mesothelioma. These include deletion of the *CDKN2A* tumor suppressor gene (>60% of cases) that encodes the cell cycle kinase inhibitor protein p16^{INK4a}, mutation of the *NF2* tumor suppressor gene (20-40% of cases) that encodes the cytoskeletal scaffolding protein Merlin, and mutation or deletion of the *BAP1* tumor suppressor gene (20-30% of cases) that encodes a deubiquitinating enzyme originally identified as a BRCA1 interacting protein.³⁻⁷ Most recently, four distinct molecular subtypes were identified that correlated with epithelioid, sarcomatoid, biphasic-epithelioid, and biphasic-sarcomatoid histologies, with the epithelioid subtype showing the longest survival times.⁷ Inactivating mutations in two epigenetic regulatory genes, *DDX3X* and *SETD2*, were identified in a 4% and 8% of pleural mesotheliomas, respectively.⁷ In addition to these somatic mutations found in sporadic mesotheliomas, germline mutation of *BAP1* has been identified in rare patients with malignant mesothelioma as part of the *BAP1* tumor predisposition syndrome (OMIM 603089), which in addition to mesothelioma includes increased incidence of uveal melanoma, meningioma, renal cell carcinoma, and other tumors.⁸⁻⁹ Given the frequent genetic inactivation of *CDKN2A* and *BAP1* in malignant pleural mesothelioma, several recent studies have assessed the sensitivity and specificity of either fluorescence in situ hybridization (FISH) or immunohistochemistry against these two genes/proteins as helpful diagnostic markers to distinguish malignant mesothelioma from benign mesothelial proliferations and metastatic carcinoma in pleural fluid.¹⁰⁻¹⁴ *BAP1* immunostaining has clearly emerged as a useful adjunct for the cytopathologist in characterizing mesothelial proliferations in the pleural cavity.

The genetic alterations which drive malignant mesothelioma in the peritoneum and other body cavities have been speculated to potentially be distinct from pleural mesotheliomas but had not been investigated until very recently. Indeed, one study performed FISH on mesotheliomas from different sites of origin and found chromosome 9p21 deletion much more commonly in pleural than peritoneal tumors (85% versus 36%), whereas 5p15 and 7p12 gains were more common in peritoneal than pleural tumors.¹⁵ A recent genome-wide analysis of 12 peritoneal mesotheliomas found *BAP1* alterations (either deletions or truncating mutations) in 7 cases, but failed to identify alterations in *CDKN2A* or *NF2* that are common in pleural mesotheliomas.¹⁶ Another recent study correlating genetic status with outcomes for patients with peritoneal mesothelioma identified frequent loss of BAP1 immunostaining that did not correlate with significant differences in outcome, but did find that tumors harboring both *CDKN2A* homozygous deletion and *NF2* hemizygous loss were associated with worse progression-free and overall survival.¹⁷ The specificity of BAP1 loss in mesothelial proliferations and histologic mimics of malignant mesothelioma in the peritoneum including serous carcinoma of the ovary is currently under investigation and has shown promising results in the limited investigations performed to date.^{12,13,18}

Given their uniformly poor prognosis, lack of well-established risk factors, and poorly understood pathogenesis, we sought to better define the genetic events that define malignant peritoneal mesothelioma and potentially identify targetable alterations to ultimately improve outcomes through a combination of next generation sequencing and immunohistochemical assays.

MATERIALS AND METHODS

Study population

This study was approved by the Institutional Review Board of the University of California, San Francisco. Thirteen cases of primary malignant peritoneal mesotheliomas were retrieved from the archives of the University of California, San Francisco Department of Pathology, spanning years 1991 to 2015. All thirteen cases were confined to the abdomen and pelvis and had no evidence of pleural involvement. All cases were re-reviewed by three gynecologic pathologists who agreed with the diagnosis of malignant mesothelioma. All 13 cases demonstrated epithelioid morphology, while one case also had a focal sarcomatoid component. Cases of multilocular peritoneal inclusion cyst also known as benign multicystic mesothelioma (n=8), well-differentiated papillary mesothelioma of the peritoneum (n=6), adenomatoid tumor (n=16), and low-grade serous carcinoma of the ovary (n=12) were also retrieved from the archives of the University of California, San Francisco Department of Pathology, spanning years 2000-2015. All specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Clinical information on patients with malignant peritoneal mesothelioma was obtained from electronic medical records. Selected histologic and clinicopathologic parameters were recorded, including patient age at diagnosis, location of tumor, type of surgical procedure, treatment, and outcome.

Capture-based Next Generation DNA Sequencing (NGS)

Genomic DNA was extracted from formalin-fixed paraffin-embedded tissue blocks of matched normal and tumor tissues of 13 patients with malignant peritoneal mesothelioma using the QIAamp DNA FFPE Tissue Kit (Qiagen). Capture-based NGS was performed at the University of California, San Francisco Clinical Cancer Genomics Laboratory, using an assay that targets the coding regions of 510 cancer-related genes, select introns of 40 genes, and *TERT* promoter with a total sequencing footprint of 2.8 Mb (UCSF500 Cancer Gene Panel). Sequencing libraries were prepared from genomic DNA. Target enrichment was performed by hybrid capture using a custom oligonucleotide library. Sequencing was performed on an Illumina HiSeq 2500. Duplicate sequencing reads were removed computationally to allow for accurate allele frequency determination and copy number calling. The analysis was based on the human reference sequence (NCBI build 37) using the following software packages: BWA, Samtools, Picard tools, GATK, CNVkit, Pindel, SATK, Annovar, Freebayes, Delly, and Nexus Copy Number.¹⁹⁻²⁶

Immunohistochemistry

Immunohistochemistry for BAP1 was performed on whole tissue sections of cases of malignant peritoneal mesothelioma (n=14), well-differentiated papillary mesothelioma of the peritoneum (n=6), multilocular peritoneal inclusion cyst (n=8), adenomatoid tumor (n=16), and low-grade serous carcinoma of the ovary (n=12). A monoclonal BAP1 antibody (clone C-4, Santa Cruz Biotechnology, sc-28383) was used at 1:100 dilution following ER2 antigen retrieval on a Leica Bond autostainer. BAP1 staining was scored as either intact (strong homogeneous nuclear staining of >90% of tumor cells) or lost (no nuclear staining in tumor cells with intact expression in non-neoplastic stromal and endothelial cells).

RESULTS

Histologic and clinical features of malignant peritoneal mesotheliomas

Thirteen cases of malignant mesothelioma confined to the abdomen and pelvis without pleural involvement were retrieved from our institutional pathology archives. These included 8 female patients (ages 19-89) and 5 male patients (ages 53-71). Twelve of the 13 cases demonstrated a predominantly epithelioid morphology, while one case had mixed epithelioid and sarcomatoid morphology. Limited clinical outcome data was available for the 13 cases, which is summarized in Table 1.

Genomic alterations in malignant peritoneal mesotheliomas

Targeted next-generation sequencing of 510 cancer-associated genes revealed pathogenic genetic alterations in each of the 13 cases (Figure 1, Supplemental Table 1). *BAP1* alterations were identified in 11 of 13 tumors (85%). Of these 11 cases, three had somatic *BAP1* mutations accompanied by loss of heterozygosity (one with a frameshift mutation, p.D362fs; one with a splice site mutation, c.68-1G>A; and one with a missense mutation, p.L97Q). One case had two somatic *BAP1* mutations (p.H669D and p.86_87del). Additionally, two cases had focal homozygous deletions of the *BAP1* locus, and two cases had structural rearrangements involving *BAP1* accompanied by loss of heterozygosity. Two

cases demonstrated heterozygous loss of the *BAP1* locus on chromosome 3p but lacked an identifiable alteration of the second allele. The final case had a germline frameshift mutation in *BAP1* (p.L573fs) that was accompanied by loss of heterozygosity in the tumor. Of the 9 cases with biallelic *BAP1* alterations, two cases also demonstrated inactivating frameshift mutations (p.V2345fs and p.Q1152fs) in the epigenetic regulatory gene *SETD2* with accompanying loss of heterozygosity. One of these tumors also harbored a truncating mutation (p.L1051fs) in the *ARID1A* gene, which encodes a subunit of the Swi/Snf chromatin remodeling complex. In addition, two other cases with biallelic *BAP1* alteration demonstrated mutations in another epigenetic regulatory gene, *DDX3X*. Three tumors harbored inactivating mutations in the *NF2* tumor suppressor gene (p.Q428*, p.F47fs, and p.L176fs) including both of the two cases with heterozygous *BAP1* loss and one case without *BAP1* alteration or chromosome 3p loss. These three truncating mutations in *NF2* were each accompanied by loss of heterozygosity. In the remaining case without *BAP1* or *NF2* alteration, a hotspot mutation in the *TERT* promoter (chr5: g.1,295,228G>A) was the solitary pathogenic alteration identified. The two tumors without *BAP1* alteration occurred in the oldest (age 85) and youngest (age 19) patients in this cohort and each demonstrated a solitary genetic alteration, *NF2* truncating mutation with loss of heterozygosity in the 85 year old and *TERT* promoter mutation in the 19 year old. The one case of peritoneal mesothelioma with mixed epithelioid and sarcomatoid morphology was the only case found to harbor homozygous deletion of *CDKN2A* and focal high-level amplification of the *MET* oncogene in addition to a structural rearrangement involving *BAP1*.

BAP1 immunohistochemistry can aid in the diagnosis of malignant peritoneal mesothelioma

BAP1 immunohistochemistry was performed on all 13 malignant peritoneal mesotheliomas (Figure 2, Table 2), as well as a series of tumors commonly considered in the differential diagnosis of malignant peritoneal mesothelioma including 6 cases of well-differentiated papillary mesothelioma, 8 cases of multicystic peritoneal inclusion cyst, 16 cases of adenomatoid tumor, and 12 cases of low-grade serous carcinoma of the ovary (Figure 3, Table 2). Loss of nuclear staining for BAP1 protein was seen in 11 of the 13 peritoneal mesotheliomas (85%), all of which demonstrated genetic alteration of the *BAP1* gene. In all 11 of these tumors, retained BAP1 expression was present in non-neoplastic stromal, inflammatory, and endothelial cells. Moreover, BAP1 loss was seen uniformly throughout all tumor cells in each case, suggesting that *BAP1* inactivation is an early or initiating event in mesothelial tumorigenesis. The absence of nuclear BAP1 protein in the two tumors with only heterozygous loss of the *BAP1* locus suggests a high likelihood of an unidentified alteration involving the second allele and potentially indicates that BAP1 immunohistochemistry may be a more sensitive diagnostic test for *BAP1* inactivation compared to capture-based next-generation sequencing assay. Intact nuclear staining for BAP1 protein was present in 2 of the 13 peritoneal mesotheliomas, both of which demonstrated two wild-type copies of *BAP1* by our sequencing assay. All 42 cases of well-differentiated papillary mesothelioma of the peritoneum, multilocular peritoneal inclusion cyst, adenomatoid tumor, and low-grade serous carcinoma of the ovary demonstrated intact BAP1 nuclear staining.

DISCUSSION

Our study reveals that while a similar spectrum of genetic alterations define both pleural and peritoneal mesothelioma, these alterations occur at distinct frequencies depending on the anatomic origin of the tumor. While *CDKN2A* deletion is very common in pleural mesotheliomas, it was only seen in one of 13 peritoneal mesotheliomas, which was the only case with a sarcomatoid component. In contrast, *BAP1* inactivation/loss is much more common in peritoneal than pleural mesotheliomas. *DDX3X* and *SETD2* mutations appear to be present in a small subset (5-15%) of both pleural and peritoneal mesotheliomas. A recent array comparative genomic hybridization study on 33 malignant peritoneal mesotheliomas identified focal deletions affecting *BAP1* (61% of cases), *NF2* (21% of cases), and *CDKN2A* (33% of cases), providing additional support for our conclusion that similar genetic alterations drive peritoneal mesothelioma but at different frequency compared to pleural mesothelioma.²⁷

Our study also provides further support for the specificity of BAP1 protein loss as a helpful diagnostic marker for the pathologic identification of malignant peritoneal mesotheliomas.¹⁰⁻¹⁴ We observed intact BAP1 expression in all 42 cases of potential histologic mimics in the abdomen and pelvis including well-differentiated papillary mesothelioma, multilocular peritoneal inclusion cyst, adenomatoid tumor, and peritoneal metastases of ovarian low-grade serous carcinoma.

Finally, our genomic profiling of malignant peritoneal mesothelioma has identified recurrent mutations in the epigenetic regulatory genes *BAP1*, *SETD2*, and *DDX3X*. These findings suggest that transcriptional deregulation is a key oncogenic mechanism in mesothelial tumorigenesis and has important therapeutic implications for the treatment of this disease. Emerging preclinical evidence suggests that inhibitors of epigenetic modifiers including histone deacetylases and the histone methyltransferase EZH2 may be efficacious in the treatment of mesothelioma.²⁸⁻²⁹ Those mesotheliomas harboring inactivating events affecting both *BAP1* and other transcriptional regulators such as *SETD2*, *DDX3X*, and *ARID1A* likely define a molecular subgroup with altered transcriptional programs that may respond favorably to these agents, compared to those mesotheliomas lacking these alterations. Additional potentially targetable alterations that emerged from our study include small molecule inhibition of MET (e.g. cabozantinib) for the subset of peritoneal mesotheliomas harboring *MET* amplification, as well as small molecule inhibition of WEE1 (e.g. AZD1775) for the subset of tumors harboring *SETD2* inactivation, a synthetic lethality that was recently reported in renal cell carcinoma and osteosarcoma.³⁰

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

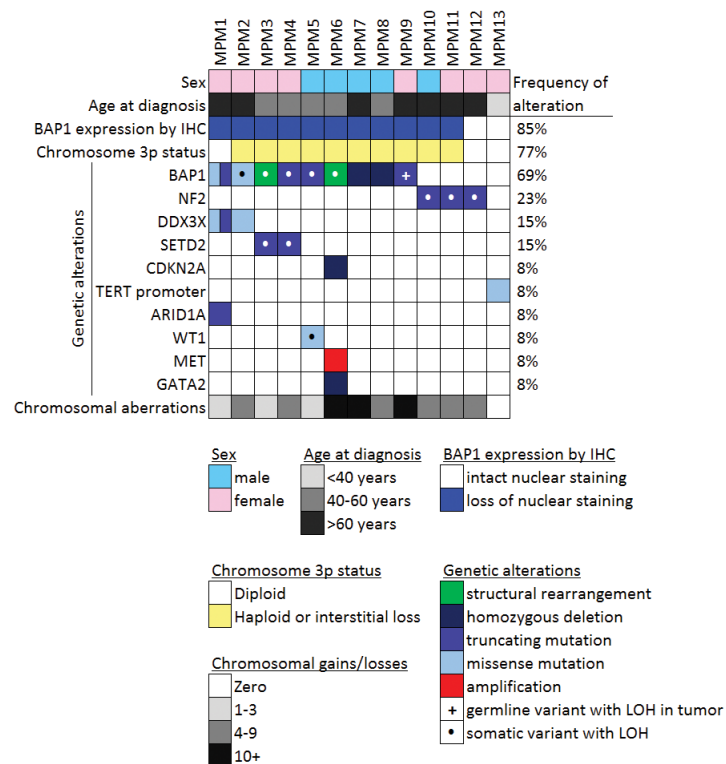
ACKNOWLEDGEMENTS

This study was funded by the UCSF Department of Pathology. D.A.S. is additionally supported by an NIH Director's Early Independence Award (DP5 OD021403).

REFERENCES

1. Robinson BWS, Lake RA. Advances in malignant mesothelioma. *N Engl J Med*. 2005; 353:1591–1603. [PubMed: 16221782]
2. Liu S, Staats P, Lee M, et al. Diffuse mesothelioma of the peritoneum: correlation between histological and clinical parameters and survival in 73 patients. *Pathology*. 2014; 46:604–609. [PubMed: 25393250]
3. Bianchi AB, Mitsunaga SI, Cheng JQ, et al. High frequency of inactivating mutations in the neurofibromatosis type gene (NF2) in primary malignant mesotheliomas. *Proc Natl Acad Sci USA*. 1995; 92:10854–10858. [PubMed: 7479897]
4. Sekido Y, Pass HI, Bader S, et al. Neurofibromatosis type 2 (NF2) gene is somatically mutated in mesothelioma but not in lung cancer. *Cancer Res*. 1995; 55:1227–1231. [PubMed: 7882313]
5. Bott M, Brevet M, Taylor BS, et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nature Genet*. 2011; 43:668–672. [PubMed: 21642991]
6. Guo G, Chmielecki J, Goparaju C, et al. Whole-exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A, and CUL1 in malignant pleural mesothelioma. *Cancer Res*. 2015; 75:264–269. [PubMed: 25488749]
7. Bueno R, Stawiski EW, Goldstein LD, et al. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. *Nature Genet*. 2016; 48:407–416. [PubMed: 26928227]
8. Testa JR, Cheung M, Pei J, et al. Germline BAP1 mutations predispose to malignant mesothelioma. *Nature Genet*. 2011; 43:1022–1025. [PubMed: 21874000]
9. Abdel-Rahman MH, Pilarski R, Cebulla CM, et al. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. *J Med Genet*. 2011; 48:856–859. [PubMed: 21941004]
10. Sheffield BS, Hwang HC, Lee AF, et al. BAP1 immunohistochemistry and p16 FISH to separate benign from malignant mesothelial proliferations. *Am J Surg Pathol*. 2015; 39:977–982. [PubMed: 25634745]
11. McGregor SM, Dunning R, Hyjek E, Vigneswaran W, Husain AN, Krausz T. BAP1 facilitates diagnostic objectivity, classification, and prognostication in malignant pleural mesothelioma. *Hum Pathol*. 2015; 46:1670–1678. [PubMed: 26376834]
12. Cigognetti M, Lonardi S, Fisogni S, et al. BAP1 (BRCA1-associated protein 1) is a highly specific marker for differentiating mesothelioma from reactive mesothelial proliferations. *Mod Pathol*. 2015; 28:1043–1057. [PubMed: 26022455]
13. Andrici J, Sheen A, Sioson L, et al. Loss of expression of BAP1 is a useful adjunct, which strongly supports the diagnosis of mesothelioma in effusion cytology. *Mod Pathol*. 2015; 28:1360–1368. [PubMed: 26226841]
14. Hwang HC, Sheffield BS, Rodriguez S, et al. Utility of BAP1 immunohistochemistry and p16 (CDKN2A) FISH in the diagnosis of malignant mesothelioma in effusion cytology specimens. *Am J Surg Pathol*. 2016; 40:120–126. [PubMed: 26448191]
15. Takeda M, Kasai T, Enomoto Y, et al. Comparison of genomic abnormality in malignant mesothelioma by the site of origin. *J Clin Pathol*. 2014; 67:1038–1043. [PubMed: 25217709]
16. Alakus H, Yost SE, Woo B, et al. BAP1 mutation is a frequent somatic event in peritoneal malignant mesothelioma. *J Transl Med*. 2015; 13:122. [PubMed: 25889843]
17. Singhi AD, Krasinskas AM, Choudry HA, et al. The prognostic significance of BAP1, NF2, and CDKN2A in malignant peritoneal mesothelioma. *Mod Pathol*. 2016; 29:14–24. [PubMed: 26493618]
18. Andrici J, Jung J, Sheen A, et al. Loss of BAP1 expression is very rare in peritoneal and gynecologic serous adenocarcinomas and can be useful in the differential diagnosis with abdominal mesothelioma. *Hum Pathol*. 2016; 51:9–15. [PubMed: 27067777]
19. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2010; 26:589–595. [PubMed: 20080505]

20. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009; 25:2078–2079. [PubMed: 19505943]
21. Broad Institute. Picard. <http://broadinstitute.github.io/picard/>
22. Talevich E, Shain AH, Botton T, Bastian BC. CNVkit: genome-wide copy number detection and visualization from targeted DNA sequencing. *PLoS Comput Biol*. 2016; 12:e1004873. [PubMed: 27100738]
23. Ye K, Schulz MH, Long Q, Apweiler R, Ning Z. Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics*. 2009; 25:2865–2871. [PubMed: 19561018]
24. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010; 38:e164–e164. [PubMed: 20601685]
25. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. *arXiv 1207.3907v2 [q-bio.GN]*.
26. Rausch T, Zichner T, Schlattl A, Stutz AM, Benes V, Korbel JO. DELLY: structural variant discovery by integrated paired-end and split-read analysis. *Bioinformatics*. 2012; 28:i333–i339. [PubMed: 22962449]
27. Chirac P, Maillet D, Lepretre F, et al. Genomic copy alterations in 33 malignant peritoneal mesothelioma analyzed by comparative genomic hybridization array. *Hum Pathol*. 2016; 55:72–82. [PubMed: 27184482]
28. Sacco JJ, Kenyani J, Butt Z, et al. Loss of the deubiquitylase BAP1 alters class I histone deacetylase expression and sensitivity of mesothelioma cells to HDAC inhibitors. *Oncotarget*. 2015; 6:13757–13771. [PubMed: 25970771]
29. LaFave LM, Beguelin W, Koche R, et al. Loss of BAP1 function leads to EZH2-dependent transformation. *Nature Med*. 2015; 21:1344–1349. [PubMed: 26437366]
30. Pfister SX, Markkanen E, Jiang Y, et al. Inhibiting WEE1 selectively kills histone H3K36me3-deficient cancers by dNTP starvation. *Cancer Cell*. 2015; 28:557–568. [PubMed: 26602815]

**Figure 1.**

Genomic profiling results on 13 patients with malignant peritoneal mesothelioma. Patient age, sex, tumor histologic subtype, chromosome 3p status, BAP1 nuclear immunostaining results, pathogenic or likely pathogenic genetic alterations, and the quantity of chromosomal copy number aberrations are shown.

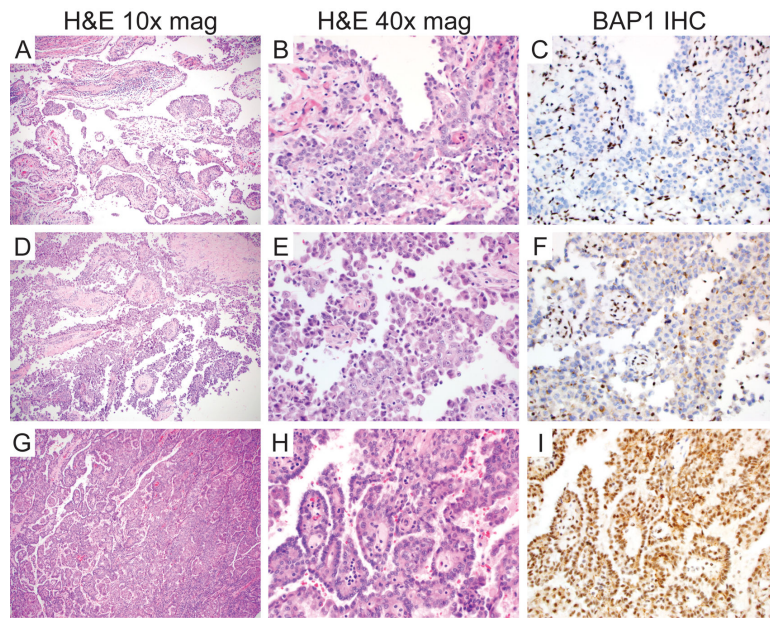


Figure 2.

Loss of nuclear BAP1 expression defines the majority of malignant peritoneal mesotheliomas arising within the abdomen and pelvis. (A-C) Absence of BAP1 immunostaining in a malignant mesothelioma arising in the abdomen/pelvis of a 70 year old woman (patient MPM11) with interstitial deletion of chromosome 3p encompassing the *BAP1* locus, as well as an *NF2* frameshift mutation. Retained BAP1 expression is seen in non-neoplastic stromal, inflammatory, and endothelial cells. (D-F) Absence of BAP1 immunostaining in a malignant mesothelioma arising in the abdomen/pelvis of a 67 year old man (patient MPM7) with focal homozygous deletion of a 300 kb segment on chromosome 3p encompassing the *BAP1* locus. (G-I) Intact BAP1 immunostaining in a malignant mesothelioma arising in the abdomen/pelvis of a 19 year old woman (patient MPM13) with hotspot mutation in the *TERT* promoter, without chromosome 3p loss or *BAP1* alteration.

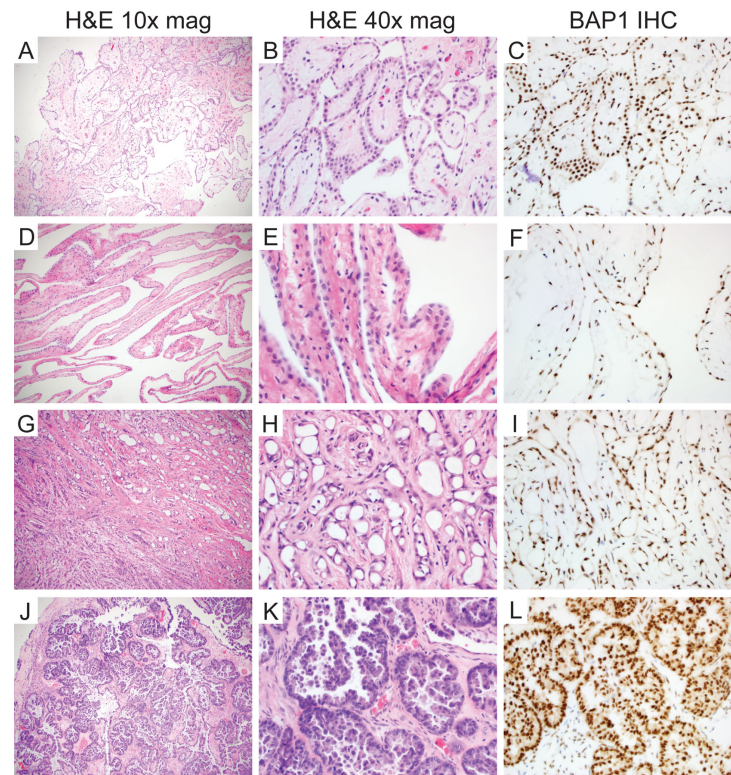


Figure 3.

Intact BAP1 expression is uniformly present in benign mesothelial lesions and other histologic mimics of malignant mesothelioma in the peritoneum. (A-C) Intact BAP1 immunostaining in a well-differentiated papillary mesothelioma within the abdomen of a 67 year old woman who presented with gastric volvulus and was found to have peritoneal nodularity along the stomach and liver. (D-F) Intact BAP1 immunostaining in an 8 cm multilocular peritoneal inclusion cyst in the pelvic cavity of a 62 year old woman. (G-I) Intact BAP1 immunostaining in a 2 cm paratesticular adenomatoid tumor in a 43 year old man who underwent orchiectomy. (J-L) Intact BAP1 immunostaining in an ovarian low-grade serous carcinoma in an 83 year old woman with multiple metastatic deposits throughout the abdomen and pelvis.

Table 1

Clinical data for patients with malignant peritoneal mesothelioma.

Patient	Age at dx	Sex	Year of dx	Presenting symptoms	Clinical outcome data
MPM1	74	F	2009	abdominal distention	diagnosed on paracentesis followed by hysterectomy/bilateral salpingo-oophorectomy/omentectomy/debulking, no follow-up available after surgery
MPM2	89	F	2008	bowel obstruction	diagnosed on bilateral oophorectomy/myomectomy/mesenteric biopsy, no follow-up available after surgery
MPM3	48	F	1996	pelvic pain	initially underwent hysterectomy/bilateral salpingo-oophorectomy with atypical mesothelial proliferation, 7 months later underwent diagnostic surgical resection of 10 cm pelvic mass and left colectomy, received intraperitoneal chemotherapy with cisplatin/doxorubicin/taxol, underwent radiation therapy, underwent two additional debulking surgeries at one and two after diagnosis, presented with bowel obstruction at three years after diagnosis due to recurrent disease, referred to hospice care without additional treatment
MPM4	47	F	2008	vaginal bleeding	diagnosed on hysterectomy/bilateral salpingo-oophorectomy/omentectomy/debulking, metastatic disease to cervical lymph nodes confirmed by biopsy at time of diagnosis, received 8 cycles of adjuvant chemotherapy with pemetrexed and cisplatin, imaging showing recurrent disease at one year after surgery, began additional treatment with gemcitabine and vinorelbine, no additional follow-up available
MPM5	48	M	1993	umbilical hernia	diagnosed on herniorrhaphy followed by debulking surgery, received adjuvant intraperitoneal chemotherapy, underwent repeat debulking surgeries at two and five years after diagnosis, no additional follow-up available after last debulking surgery
MPM6	54	M	2004	abdominal distention	diagnosed on paracentesis, received 6 cycles of neoadjuvant chemotherapy with pemetrexed and cisplatin, then underwent debulking surgery with right colectomy, no follow-up available after surgery
MPM7	67	M	1991	abdominal wall mass	diagnosed on resection of mass and involved small bowel, no follow-up available after surgery
MPM8	55	M	2000	abdominal distention and ascites	initially had five non-diagnostic paracentesis cytologic evaluations, diagnosed on laparoscopy with omental biopsy followed by omentectomy and surgical debulking, no follow-up available after surgery
MPM9	62	F	2014	pelvic mass	diagnosed on hysterectomy/bilateral salpingo-oophorectomy/omentectomy/colectomy, no follow-up available after surgery
MPM10	71	M	2002	abdominal distention	diagnosed on omentectomy and debulking surgery, no follow-up available after surgery
MPM11	70	F	2014	abdominal distention and ascites	diagnosed on laparoscopy with omental biopsy, no follow-up available after biopsy
MPM12	85	F	2006	abdominal distention and ascites	diagnosed on hysterectomy/bilateral salpingo-oophorectomy/omental biopsy, no follow-up available after surgery
MPM13	19	F	2004	abdominal distention	diagnosed on bilateral salpingo-oophorectomy/omentectomy/surgical debulking, received adjuvant chemotherapy with 4 cycles of pemetrexed and cisplatin, developed recurrent disease four years after diagnosis, received 3 cycles of pemetrexed and carboplatin, underwent hysterectomy and surgical debulking, treated with gemcitabine then vinorelbine then liposomal doxorubicin, died of disease six years after diagnosis

Table 2

BAP1 immunostaining results on malignant peritoneal mesotheliomas and histologic mimics in the peritoneum.

Tumor type	Cases with loss of nuclear BAP1 immunostaining	Total number of cases	% with BAP1 loss
Malignant peritoneal mesothelioma	11	13	85%
Ovarian low grade serous carcinoma	0	12	0%
Well-differentiated papillary mesothelioma	0	6	0%
Multilocular peritoneal inclusion cyst	0	8	0%
Adenomatoid tumor	0	16	0%